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Secretion of Mitogenic Factor(s) from Stocks of *Paramecium tetraurelia*, *P. caudatum* and *P. multimicronucleatum*

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ABSTRACT—We recently isolated and purified the Paramecium growth factor (ParGF). The starting material (crude sample) was a ca.100-fold concentrate obtained by ultrafiltration of a cell-free medium of an early stationary mass culture of the *jumyo* mutant of *Paramecium tetraurelia*. Its mitogenic function has been assessed from the restoration of the reduced fission rate of the *jumyo* mutant in daily reisolation cultures. To determine whether stocks of *P. tetraurelia* other than the *jumyo* mutant and those of other *Paramecium* species secrete similar substance(s), we assayed crude samples of those stocks for mitogenic function using the *jumyo* mutant. The results obtained were positive for almost all the stocks of *P. tetraurelia*, *P. caudatum* and *P. multimicronucleatum* studied, suggesting that ParGF, or a functional homologue, is common to *Paramecium* and act as a mitogen on "non-self" cells as well as on "self" cells (i.e. those producing the factor).

INTRODUCTION

Paramecium cells divide 3–5 fissions a day at 25°C in bacterized Wheat-Grass-Powder medium. The *jumyo* mutant [3], which was first isolated as a mutant having a short clonal lifespan [4], divides 0.5–1.5 fissions a day in daily reisolation cultures but 3–5 fissions a day in mass culture. This characteristic of the mutant provided the clue that led to the finding of Paramecium growth factor (ParGF) and to a method for its biological assay [5]. ParGF has been collected and purified from a cell-free medium of an early stationary-phase culture of *jumyo* cells and has been assayed in daily reisolation cultures of these cells. We investigated whether the cell-free medium derived from stocks of *P. tetraurelia* other than the *jumyo* mutant and those of other *Paramecium* species can restore the reduced fission rate of the *jumyo* mutant in daily reisolation culture.

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MATERIALS AND METHODS

Stocks

All the stocks except for G3 were from the collections of Y.T. at Nara Women's University. Figure 1 shows the lineages of 12 stocks of *P. tetraurelia* as well as the mating types for some stocks, the genotypes of the *jumyo* locus [3] and the *nd169* locus [1] for all the stocks. Stocks G3 (mating type V) and NK15 (VI) of *P. caudatum* syngen 3 and stocks CH313 (III) and CH312 (IV) of *P. multimicronucleatum* syngen 2 were used. G3 was provided by Dr. M. Fujishima of Yamaguchi University.

Culture

Cells from a given stock were washed and cloned according to the method of Sonneborn [2]. The culture medium was a 1% phosphate-buffered medium of 5 g/l Wheat-Grass-Powder (Pines International, Inc., USA), inoculated with *Klebsiella pneumoniae* 1–2 days before use.

For the mass culture, 1,000 ml flasks, each

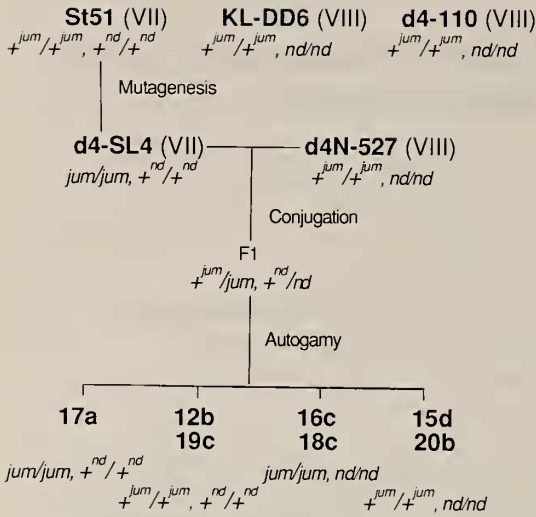

FIG. 1. Lineages of *P. tetraurelia* stocks with their mating types and genotypes of the *jumyo* and *nd169* loci. The two genes are abbreviated *jum* and *nd*. d4-SL4 is the *jumyo* mutant.

containing 400 ml culture medium, were used. After introduction of 1–10 cells/ml, the individual flasks were incubated at 26°C as still cultures. When the culture first became clear, indicating exhaustion of bacteria, it was considered to be at the early stationary-phase of growth.

For the daily reisolation culture, a single cell was placed in 400 μl of culture medium in a well of a depression slide housed in a moist chamber.

Preparation of the crude sample

Cells in the early stationary-phase culture were removed by filtering the medium through two folds of two sheets of filter paper (TOYO). The resulting cell-free fluid was concentrated about 100-fold by ultrafiltration in a Diaflow cell (Amicon) with a Diaflow ultrafiltration membrane YM10 (Amicon) that had a nominal cut-off molecular weight of 10,000. The crude sample obtained was stored in a freezer (−25°C) and thawed in a refrigerator before use.

Assay of the crude sample for mitogenic function

Cells were taken from a clonal culture of the *jumyo* mutant and placed in 12 (or 18) wells of depression slides, each containing 400 μl of culture medium, then cultured as daily reisolation lines for 5 days, half the lines being designated experimental and half control. For each experimental culture, a crude sample was added daily so as to raise concentration 1.5-fold of that in the original cell-free culture medium (when the sample was a 100-fold concentrate, 6 μl was added; an 80-fold concentrate, 7.5 μl was added). When a line died out, it was replaced by one of the other living lines.

Fission rate was compared between two groups of lines for both the daily averages and the total averages on the 5th day. The crude sample was considered effective as a mitogen when the total average of the daily fission rates in the experimental group was significantly higher (*t*-test).

RESULTS

Secretion of mitogenic factor from stocks of P. tetraurelia other than the jumyo mutant

Of the 12 stocks of *P. tetraurelia* with various combinations of mating types and the genotypes of the *jumyo* and *nd169 loci* (see Fig. 1), 11 stocks (except 17a) were examined for the secretion of mitogenic factor, or a functional homologue of ParGF. The crude sample collected from the early stationary-phase culture of each stock effectively restored the fission rate of *jumyo* cells in daily reisolation culture. The results for 6 representative stocks with various combinations of mating types and genotypes are shown in Figure 2. The lines of *jumyo* cells supplemented with the crude sample from the indicated stock showed significantly higher fission rates than unsupplemented control lines, although the restored level of the fission rate was not so high as the wild-type level. The crude sample prepared from bacterized medium that had *not* been inoculated with *Paramecium* cells showed no such effect (data not shown).

These results indicate that the ability to secrete mitogenic factor, probably ParGF or a homologue, has nothing to do with differences in mating types and genotypes of the *jumyo* and *nd169* loci.

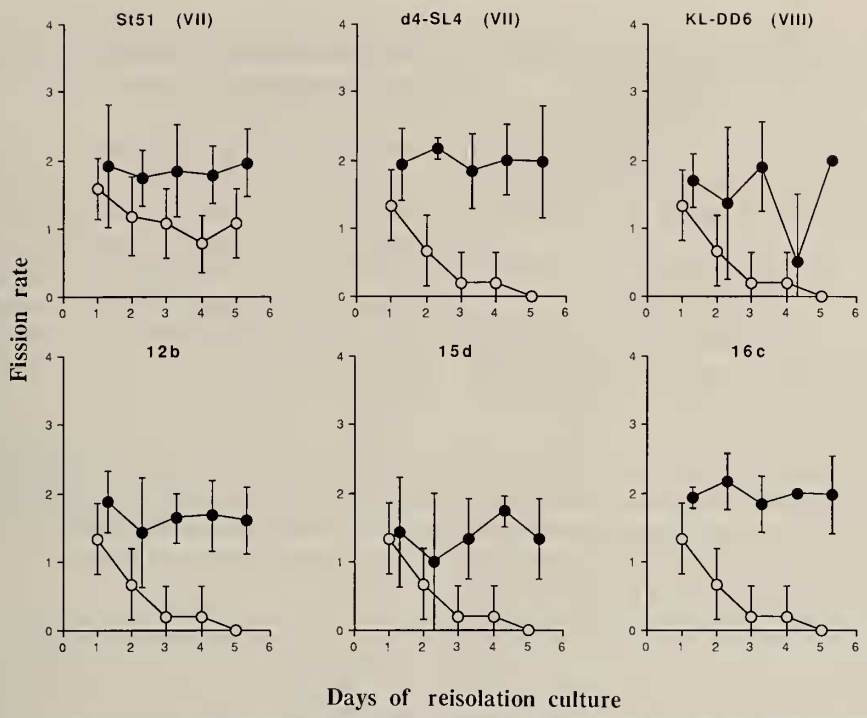

FIG. 2. Mitogenic activity of crude samples prepared from *P. tetraurelia* stocks with various genetic backgrounds (see Fig. 1). Controls (open circles) are for 6 or 9 daily reisolation lines of *jumyo* cells cultured in depression slide wells that contained 400 μl of culture medium. Experiments (closed circles) are for 6 or 9 daily reisolation lines supplemented daily with the crude sample prepared from the stock indicated. Daily reisolation cultures were begun on day 0 and maintained for 5 days. The fission rate is given in $\log_2 N$ where N is the number of cells on the day following reisolation. Bars indicate standard deviations.

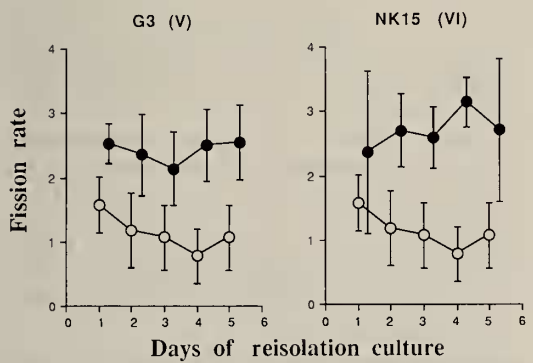

FIG. 3. Mitogenic activity of crude samples prepared from *P. caudatum* stocks of different mating types. Same as in Figure 2.

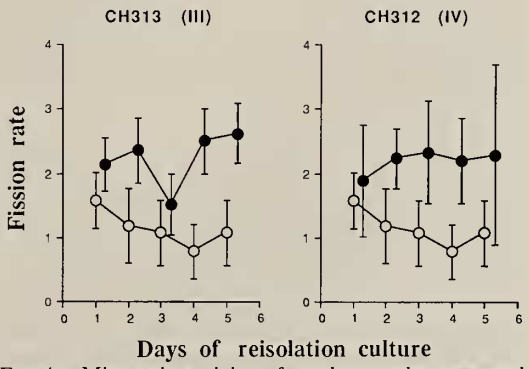

FIG. 4. Mitogenic activity of crude samples prepared from *P. multimicronucleatum* stocks of different mating types. Same as in Figure 2.

restored the fission rate of *jumyo* cells in daily reisolation culture. These results strongly suggest that the mitogenic factor, probably ParGF or a homologue, is secreted into the surrounding medium from every stock of *Paramecium* species.

Secretion of mitogenic factor from stocks of P. caudatum and P. multimicronucleatum

The crude sample collected from the early stationary-phase culture of two stocks of *P. caudatum* (Fig. 3) and two of *P. multimicronucleatum* (Fig. 4), all of different mating types, effectively

DISCUSSION

ParGF first was identified by the use of the *jumyo* mutant both as a donor in mass culture and as a recipient in daily reisolation cultures for the assay [5]. ParGF therefore has been regarded as a substance recognized by "self" cells. This study shows that mitogenic factor(s) is secreted not only by a variety of *P. tetraurelia* cells which are of different mating types and are homozygous dominant and recessive for the *jumyo* locus (Figs. 1, 2), but also by cells with different mating types of *P. caudatum* (Fig. 3) and *P. multimicronucleatum* (Fig. 4). Since what we have tested for the mitogenic activity in this study is not the purified ParGF but crude samples, the restoration of the reduced fission rate of *jumyo* cells is not due to ParGF itself but to interaction of molecules present in the crude samples. However, it is highly probable that a functional homologue of ParGF is secreted by a number of *Paramecium* species and acts on *jumyo* cells; ParGF may be a substance that is recognized by "non-self" cells as well as by "self" cells and all *Paramecium* cells may produce both ParGF and its receptor.

The fact that the *jumyo* mutant divides slowly in daily reisolation culture, where the substances secreted are abandoned daily, but divides rapidly in mass culture, where the substances secreted are accumulated, suggests that the mutant may be slow either in the rate of production of ParGF or in the effectiveness to respond to ParGF, or both. Although we repeatedly prepared crude samples from the same stock and tested their mitogenic activity with the use of this assay method, the levels of the fission rate restored were not consistent and thus comparison of the kinetics among different stocks was impossible. Therefore, the above-mentioned possibilities both remain open. For that, we need the improvement of the assay method that should be accurate enough to compare the quantity and quality of ParGF as well as the use of purified ParGF.

We are so far unsuccessful in developing a new assay method in which wild type cells, not *jumyo* mutant cells, are used. When wild type cells are cultured in a bacterized medium in which Wheat-Grass-Powder is reduced to 1/16 that of the standard, they divide as slowly as *jumyo* mutant cells in daily reisolation cultures. But, this low fission rate can be restored not only by crude samples but also by concentrates of bacterized medium in which *Paramecium* cells had not been inoculated (data not shown). Therefore, the low fission rate induced by nutritional restriction cannot represent the low fission rate of the *jumyo* mutant in daily reisolation culture.

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